FLUORESCENT ANTIBODIES FOR THE DETECTION OF RICKETTSIAE
(REVIEW OF THE LITERATURE)

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Following is the translation of an article by N. M. Balayeva, Ye. N. Levina and M. Ya. Korn, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) # 3, 1965, pages 17--21. It was submitted on 11 Mar 1964. It was presented at the 7th International Congress on Problems of Tropical Medicine and Malaria at Rio-de-Janeiro in 1963. Translation performed by Sp/7 Charles T. Ostertag, Jr.

The detection of rickettsiae in various materials and their identification are connected with a number of difficulties. For these purposes up until the present time it was possible to use only complex immunobiological and serological methods which took a long time to carry out. In some cases the establishment of the rickettsia nature of a causative agent was made more complex by the necessity of preparing antigen from the isolated microorganisms. In connection with this the interest and attention paid to the luminescent-serological method of investigation by investigators working in the field of rickettsioses is understandable. This method, combining a morphological and serological appraisal, makes it possible to judge concerning the nature of the causative agent or to observe it during the course of the development of the pathological process. Besides this, it turned out to be suitable for the serological investigations during rickettsial diseases with the purpose of exposing antibodies in the blood of the patients.

In 1950 Coons and coauthors for the first time showed the principle feasibility of exposing Rickettsia prowazeki and Rickettsia rickettsi by the direct method of fluorescent antibodies. Since this time a significant number of investigations have been published on the use of the direct (Coons et al., 1942, 1950) and the indirect (Weller and Coons, 1954; Goldwasser and Shepard, 1958) method of luminescent-serological detection of rickettsiae. These make it possible to make a judgment on the feasibilities of this method. It was established by these investigations that the detection of rickettsiae by the direct method of fluorescent antibodies is highly specific and sufficiently sensitive. With the help of luminescent sera it is possible to quite clearly differentiate rickettsiae which are remote in antigenic structure (Coons et al., 1950; Balayeva et al., 1960, 1962, Krasnik et al., 1960).
Thus, luminescent sera, prepared for rickettsiae of the typhus group, did not react with rickettsiae from the tick-borne spotted fever group and *Rickettsia burneti*. The sera to *Rickettsia burneti* permit the exposure only of the causative agent which is homologous for them. In order to differentiate rickettsiae which are close in antigenic structure, for example *Rickettsia prowazeki* and *Rickettsia mooseri*, the preliminary sorbtion of the sera with the corresponding antigens is necessary.

In contrast to the direct method the indirect method is two-phased: In the first phase the preparation is treated with immune unlabeled serum, in the second -- with antispecies luminescent serum. Therefore, the defects of the luminescent-serological method, depending on the increase of the sorbtion capability of the rickettsiae and the tissues following fixation on glass and leading to the nonspecific sorbtion of serumal proteins, increase with the indirect method. Here the nonspecifically sorbed proteins of unlabeled serum are exposed then by the luminescent antispecies serum (Balayeva, et al., 1962). The greatest number of investigations on the detection of rickettsiae with the help of the luminescent-serological method were conducted in respect to rickettsiae of the typhus and Q-fever group. It was established that rickettsiae of the typhus group, especially *Rickettsia prowazeki*, may be detected in clothes lice, the organs of infected animals, chick embryos (Coons et al., 1950; Goldwasser and Shepard, 1959; Levina and Balayeva, 1960; Balayeva et al., 1962; Krasnik et al., 1960; Khavkin and Amosenkova, 1963), tissue cultures (Riggs, et al., 1958; Krasnik, 1963) and blood smears (Balayeva and Nikolskaya, 1962).

There is special significance in the investigations on the exposure of *Rickettsia prowazeki* in the carrier of typhus - clothes lice. According to the data of Krasnik and Goldin (1960), with the help of luminescent sera it is possible to very clearly differentiate *Rickettsia prowazeki* in clothes lice from *Rickettsia quintana* and *Rickettsia rocha-limae*, which are also inhabitants of the intestines of clothes lice. Here it is possible to detect all the morphological forms of development of the causative agent in the lice, beginning with filiform and ending with coccoid (Balayeva et al., 1962).

The feasibility of the detection of rickettsiae in the carrier and the reliable differentiation of them from other rickettsiae make it possible to recommend the use of this method in epidemiological practice for the rapid establishment of the nature of the rickettsiae detected.

The method of luminescent antibodies also opens new possibilities for clearing up unclear problems in the pathogenesis of rickettsioses. Thus, the use of the method of luminescent antibodies with the aim of detecting *Rickettsia prowazeki* in the blood made it possible to show (Balayeva and Nikolskaya, 1962) that following the administration of a
toxic suspension of *Rickettsia prowazeki* to mice, the rickettsiae were detected in smears of their blood in just 1--2 minutes, and then disappeared from the blood stream. These observations have significance for the interpretation of the pathogenesis of rickettsial intoxication.

In studying the specificity of the method of luminescent antibodies, data were obtained which are of interest from the point of view of the antigenic interrelationships between rickettsiae and proteus OX19 (Balayeva et al., 1962). It turned out that the result of staining proteus with luminescent sera against *Rickettsia prowazeki* depends on the method of preparing the sera: Sera, obtained from rabbits which were infected one time with a culture of *Rickettsia prowazeki*, stain proteus, and sera from hyperimmune animals lose this capability. The data obtained are found in conformity with the old observations of Otto and Munter (1930) concerning the disappearance, in rabbit sera, of the ability to react in the Weil-Felix reaction following their repeated infection with *Rickettsia prowazeki*, and also with the more recent analogous data of Goldberg (1952), Krestovnikova and Zhurbina (1949).

Attention is merited by the fact (Balayeva et al., 1962) that in the event of a positive staining of proteus with luminescent serum against *Rickettsia prowazeki*, analogous results were fixed during the staining of the cultures with the preserved and destroyed (by prolonged boiling) surface antigen. Consequently, it is apparent that the antigens of proteus OX19, which are common with the antigens of *Rickettsia prowazeki*, are disposed not just superficially.

The method of luminescent antibodies has found wide application for the detection of *Rickettsia burneti*. For the first time it was used simultaneously by Roberts and Downs (1959) for observing the development of *Rickettsia burneti* in a tissue culture and by Goldin and Amosenkova (1961) for detecting *Rickettsia burneti* in various cultures. The significantly expressed rickettsiemia during Q-rickettsiosis made it possible to successfully use the method of fluorescent antibodies for the direct detection of *Rickettsia burneti* in smears of blood from experimentally infected animals. The authors also reported of the positive results of using this method for the purpose of investigating persons afflicted with Q-rickettsiosis. In blood smears, treated with fluorescent Q-rickettsial serum, rickettsiae were detected in the form of minute coccoid-bacillary formations, disposed on the surface of erythrocytes. The stated method also made it possible to considerably shorten the period for the identification of *Rickettsia burneti* upon the isolation of the causative agent from humans and animals: Already in 3--7 days following the infection of animals with material in which the presence of *Rickettsia burneti* is assumed, they can be identified in smear-prints, while with the help of the ordinary immunobiological methods of investigation an answer may be obtained in no less than 20--25 days from the onset of the infection.
The method of fluorescent antibodies may also be used for the detection of *Rickettsia burneti* in ticks (Goldin and Amosenkova, 1961), where they were exposed for a period of 8 months following infection (the period of observation). Here the brightness of fluorescence of the rickettsial cell did not depend on the biological condition of the tick.

In respect to *Rickettsia rickettsi* the main attention of the investigators was directed at their exposure in ticks, in which along with *Rickettsia rickettsi* there were a large number of organisms which were similar to rickettsiae. To differentiate these organisms from *Rickettsia rickettsi* could be done only by means of immunobiological methods up until the present time. Shepard and Goldwasser (1960), in using the method of fluorescent antibodies for this purpose and making a comparison of it with the biological method of isolating rickettsiae by means of infecting chick embryos and guinea pigs, came to a conclusion concerning the great sensitivity of the luminescent-serological method. It turned out that in the majority of ticks the rickettsiae, detected with the help of luminescent serum, were infectious for just the chick embryos, or for the embryos and the guinea pigs. However, in a number of cases rickettsiae, determined by luminescent serum, were not infectious either for chick embryos or for guinea pigs, but they impart to the latter an immunity against *Rickettsia rickettsi*. The authors explain this by the presence of nonviable rickettsiae in the ticks.

Burgdorfer and Lackman (1960) and Burgdorfer (1961) turned attention to the dependency of the intensity of the fluorescence of *Rickettsia rickettsi* on the biological condition of the ticks; in young ticks and gorged adults the fluorescence of the rickettsiae is bright, the rickettsiae appear as rod-shaped and thread-like forms, and in adult hungry ticks the rickettsiae have a spherical form and fluoresce weakly; following the feeding of the ticks the rickettsiae again take on an intensively luminescent rod-shaped form. The authors explain the appearance of atypical forms of rickettsiae by the influence of unfavorable conditions on the microorganisms and express an assumption concerning a bond between this phenomenon and a change in the virulence of the rickettsiae.

Krasnik and Goldin (1961) reported of the positive results in the application of luminescent antibodies for the exposure of *Rickettsia quintana* in fleas, and Winsen and Fuller (1961) -- in lice, chick embryos and cell cultures. It can be assumed that these observations will help simplify the diagnosis of Volhynia rickettsiosis.

Such are the main results in the application of the method of luminescent antibodies for the exposure and identification of rickettsiae. Of particular importance here are the data concerning the feasibility of detecting rickettsiae in the carrier (*Rickettsia prowazekii* and *R. quintana* in clothes lice, and *R. rickettsi* and *R. burneti* in ticks). The method of
Luminescent antibodies is an unique method for the mass investigation of carriers for their natural infectious state with rickettsiae, which can hardly be resolved with the help of biological methods of investigation. It may also be used for the detection of antibodies in the blood during rickettsial infections, especially in typhus. For these purposes the indirect methods of luminescent-serological analyses of Weller and Coons, and Goldwasser and Shepard (1958, 1959) are used. By this method it is possible to detect the antibodies by setting up the reaction with antigen in test tubes or on glass. The latter significantly simplifies the investigations and shortens their duration.

The isolation of antibodies by this method requires the observance of specific conditions, connected with the peculiarities of the indirect method of the luminescent-serological detection of rickettsiae. The rickettsial cell, which is fixed on glass, possesses the capability to sorb serumal proteins, not only based on the bond of the antigen-antibody, but on the strength of physico-chemical interactions. In order to reduce the influence of the latter, special methods have been developed: The use of sparing fixation of the rickettsial antigen on glass, in particular by means of ethyl alcohol (96%), the application of sera, inactivated for 30 minutes at 56°C in dilutions with a physiological solution having an alkali reaction (pH 7.2-9.0), the use of sera in dilutions of 1:20-1:40, and the employment of anti-species sera, sorbed by tissue powders. In carrying out these requirements, the detection of antibodies to Rickettsia prowazeki in the sera of typhus patients with the help of the luminescent-serological method is sufficiently specific. The titers of the antibodies in the sera, determined by this method, depend on the activity of the anti-species sera and fluctuates within the limits of the titers of antibodies, established in the compliment fixation reaction (Balayeva et al., 1960, 1963).

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